

index phenotype displayed by the index inbred strain, thereby indicating that at least one F₁ individual possesses an index phenotype-modifying mutation;

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Cont backcrossing gametes from male F₁ progeny to at least one female of the index inbred strain, with or without the index allele, to obtain N₂ backcross progeny, wherein at least one of the N₂ backcross progeny that carry the dominant allele also exhibit the outlying phenotype; and

verifying that the outlying phenotype is caused by a segregating single point mutation.

2. A method as claimed in Claim 1 wherein any of the crosses employ preserved gametes.

3. A method as claimed in Claim 1 wherein the F₁ progeny and some of the N₂ progeny exhibit an extreme outlying phenotype.

4. A method as claimed in Claim 3 wherein the segregating mutation is a heterozygous modifier of the index phenotype selected from a group consisting of an enhancing modifier and a suppressing modifier.

5. A method as claimed in Claim 1 wherein the dominant allele is a *Min* allele at an *Apc* locus in a mouse.

6. A method as claimed in Claim 1 wherein the index inbred strain and the founder inbred strain share an isogenic genetic background.

7. (Amended Two Times) A method as claimed in Claim 6 further comprising the step of mapping the segregating mutation using a mapping partner strain, the mapping partner strain being produced by the steps of:

e2 treating an animal of the founder strain with a mutagenic agent to induce point mutations in the treated animal;

crossing the treated animal to an animal of the founder strain to produce F₁ progeny; and

sib-mating F₁ and subsequent generation progeny until detrimental and lethal mutations are eliminated.

8. A method as claimed in Claim 1 wherein the founder inbred mouse strain is produced by a method comprising the step of treating a wild-type inbred mouse with a mutagenic agent to induce point mutations.

9. A method as claimed in Claim 8 wherein the mutagenic agent is ethylnitrosourea.

10. A method for identifying a human genetic sequence that corresponds to a segregating mutation at a genetic locus in a non-human animal, the segregating mutation causing an outlying phenotype relative to an index phenotype in an index inbred mouse strain, the method comprising the steps of:

outcrossing a founder inbred non-human strain to an index inbred non-human strain to obtain F₁ progeny, the founder inbred strain carrying random point mutations relative to a wild-type animal of the founder inbred strain, the index inbred strain carrying a dominant allele at a locus known to confer the index phenotype and being genetically distinguishable from the founder inbred strain, wherein some of the F₁ progeny that carry the dominant allele also carry at least one random mutation;

backcrossing the F₁ progeny to the index inbred strain, with or without the index allele, to obtain N₂ backcross progeny, wherein at least some of the N₂ backcross progeny that carry the dominant allele also exhibit the outlying phenotype;

verifying that the outlying phenotype is caused by a segregating mutation;

identifying genetic markers linked to the segregating mutation;

identifying a gene on a contig that encodes the segregating mutation; and

recovering human genetic sequences that correspond to the mutation-encoding gene.

11. A method for identifying a segregating mutation at a genetic locus that modifies an index phenotype in a non-human index inbred strain, the segregating mutation causing an outlying phenotype relative to the index phenotype, the method comprising the steps of:

crossing a non-human founder inbred strain with a non-human index inbred strain to obtain Gen₁ progeny, the founder inbred strain carrying random point mutations relative to a wild-type animal of the founder inbred strain, the index inbred strain carrying a congenic dominant allele at a locus known to confer the index phenotype, the founder strain and the

index strain sharing an isogenic genetic background, wherein some of the Gen1 progeny that carry the dominant allele also exhibit a modified index phenotype; and

verifying that Gen1 progeny that carry the dominant allele and exhibit a modified index phenotype carry a segregating mutation.

12. A method as claimed in Claim 11 wherein the genetic background has no modifying effect upon the index phenotype.

13. A method as claimed in Claim 11 wherein the genetic background has a modifying effect upon the index phenotype.

14. A method as claimed in Claim 13 wherein the genetic background has an enhancing effect upon the index phenotype, and wherein the Gen1 animals exhibit a suppressed phenotype relative to the index inbred strain.

15. A method as claimed in Claim 11 further comprising the steps of:
mapping the segregating mutation by crossing Gen1 animals that have the dominant allele and a modified index phenotype to a genetically distinguishable inbred strain; and
evaluating the progeny of the mapping cross.

16. A method as claimed in Claim 15 wherein the genetically distinguishable inbred strain shares an isogenic genetic background with the founder and index strains and further comprises single nucleotide polymorphisms relative to the founder inbred strain.

17. A genetically altered mouse comprising in its genome:
a congenic dominant heterozygous allele that confers an index phenotype on the mouse;
a segregating modifier of the index phenotype, the modifier being attributable to a single point mutation, and
a single nucleotide mapping polymorphism genetically linked to the single point mutation.

18. A mouse as claimed in Claim 17 wherein the dominant allele is a *Min* allele at an *Apc* locus.

19. A non-human animal comprising a segregating mutation that modifies an index phenotype, the animal being prepared according to a method comprising the steps of:

outcrossing at least one male animal of a founder inbred non-human strain to at least one female animal of an index inbred non-human strain to obtain F₁ progeny, the founder inbred strain carrying random point mutations relative to a wild-type animal of the founder inbred strain, the index inbred strain carrying a congenic dominant allele at a locus known to confer the index phenotype and being genetically distinguishable from the founder inbred strain, wherein at least one of the F₁ progeny that carry the dominant allele also carry at least one random mutation;

backcrossing gametes from male F₁ progeny to the index inbred strain, with or without the index allele, to obtain N2 backcross progeny, wherein at least one of the N2 backcross progeny that carry the dominant allele also exhibit the outlying phenotype;

verifying that the outlying phenotype is caused by a segregating mutation; and
selecting an animal that shows the outlying phenotype.

20. A non-human animal as claimed in Claim 19 wherein the non-human animal is a mouse.

21. A non-human animal comprising a segregating mutation that modifies an index phenotype, the animal being prepared according to a method comprising the steps of:

crossing a founder inbred strain with an index inbred strain to obtain Gen1 progeny, the founder inbred strain carrying random point mutations relative to a wild-type animal of the founder inbred strain, the index inbred strain carrying a congenic dominant allele at a locus known to confer the index phenotype, the founder strain and the index strain sharing an isogenic genetic background, wherein some of the Gen1 progeny that carry the dominant allele also exhibit a modified index phenotype;

verifying that Gen1 progeny that carry the dominant allele and exhibit a modified index phenotype carry a segregating mutation; and

selecting an animal that shows the outlying phenotype.

22. A non-human animal as claimed in Claim 21 wherein the non-human animal is a mouse.

23. (Amended) A non-human animal comprising a segregating mutation that modifies an index phenotype, the animal being prepared according to a method comprising the steps of:

outcrossing a founder inbred strain with the index inbred strain to obtain Gen1F₁ progeny, the founder strain being heterozygous only for random point mutations relative to a wild-type animal of the founder inbred strain, the index inbred strain carrying a dominant allele at a locus known to confer the index phenotype, where at least some of the Gen1F₁ progeny carry both the dominant allele and at least one random mutation;

e3 crossing a founder animal of the founder inbred strain to an animal of the founder strain that lacks the mutations to obtain Gen2 offspring, where the founder animal has at least one outcrossed F₁ progeny that displays the outlying phenotype relative to the index phenotype;

outcrossing Gen2 offspring to the index strain to obtain Gen2F₁ backcross progeny, half of which, on average, carry the dominant allele that confers the index phenotype; and verifying that a subset of the Gen2F₁ progeny shows the outlying phenotype; and selecting an animal that shows the outlying phenotype.

24. A non-human animal as claimed in Claim 23 wherein the non-human animal is a mouse.

25. (Amended) A method for identifying a segregating mutation at a genetic locus that modifies an index phenotype in a non-human index inbred strain, the segregating mutation causing an outlying phenotype relative to the index phenotype, the method comprising the steps of:

e4 outcrossing a non-human founder inbred strain with the non-human index inbred strain to obtain Gen1F₁ progeny, the founder strain being heterozygous only for random point mutations relative to a wild-type animal of the founder inbred strain, the index inbred strain carrying a dominant allele at a locus known to confer the index phenotype, where at least some of the Gen1F₁ progeny carry both the dominant allele and at least one random mutation;

crossing a founder animal of the founder inbred strain to an animal of the founder strain that lacks the mutations to obtain Gen2 offspring, where the founder animal has at least one outcrossed F₁ progeny that displays the outlying phenotype relative to the index phenotype;

24
outcrossing Gen2 offspring to the index strain to obtain Gen2F₁ backcross progeny, half of which, on average, carry the dominant allele that confers the index phenotype; and verifying that a subset of the Gen2F₁ progeny shows the outlying phenotype.

26. (Amended) A method as claimed in Claim 6 wherein the method identifies a segregating mutation at a genetic locus that modifies tumor multiplicity in a C57BL/6 mouse congenic for the *Min* allele at the *Apc* locus, the method comprising the steps of:

outcrossing at least one male C57BL/6 mouse carrying random point mutations to a female C57BL/6 mouse congenic for the *Min* allele at the *Apc* locus to obtain F₁ progeny;

identifying one or more F₁ individuals displaying an outlying tumor multiplicity phenotype relative to the tumor multiplicity phenotype in a C57BL/6 mouse congenic for the *Min* allele at the *Apc* locus, thereby indicating that at least one F₁ individual possesses a segregating mutation that modifies tumor multiplicity; and

backcrossing gametes from male F₁ progeny to at least one female C57BL/6 mouse congenic for the *Min* allele at the *Apc* locus to obtain N2 backcross progeny, wherein at least one of the N2 backcross progeny carries the *Min* allele and has a tumor multiplicity that is modified relative to tumor multiplicity in a C57BL/6 mouse congenic for the *Min* allele at the *Apc* locus, the modified tumor multiplicity being characteristic of the segregating mutation thereby identified.

27. (Amended Two Times) A method as claimed in Claim 26 wherein the modified tumor multiplicity is evaluated according to a method comprising the steps of:

repeatedly applying for random permutations of mice among N2 backcross subkindreds a likelihood ratio test of the null hypothesis that no multiplicity modifier is segregating to obtain a p-value, wherein a p-value of less than 0.05 indicates a potential carrier of the segregating mutation;

when the p-value is less than 0.05, calculating, for each potential carrier that has offspring with information about tumor multiplicity, a LOD score for presence of the segregating mutation, wherein the LOD score is log₁₀ of a ratio of a first probability of offspring phenotype data if the potential carrier mouse carries a multiplicity modifier to a second probability of offspring phenotype data if the potential carrier mouse does not carry a multiplicity modifier, and wherein the second probabilities are calculated from an estimated background distribution and the first probabilities are calculated from a mixture of the estimated background distribution and an estimated modified distribution, where the

estimated distributions are obtained by a maximum likelihood method; and
ranking the LOD scores of potential carriers, whereby animals having the highest
LOD scores are likely carriers of the segregating mutation.

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Amended* 28. (Amended Two Times) A method as claimed in claim 26, further comprising
the step of mapping the segregating mutation in the N2 backcross progeny using a mapping
partner strain having a C57BL/6 genetic background.

29. A method as claimed in Claim 28 wherein the mapping partner strain is
produced by the steps of:
treating a C57BL/6 mouse with a mutagen to introduce random point mutations;
crossing the treated mouse to a C57BL/6 mouse to produce F1 progeny; and
sib-mating F1 and subsequent generation progeny until detrimental and lethal
mutations are eliminated.

30. (Amended) A method for identifying a segregating single point mutation at a
genetic locus that modifies an index phenotype in a mouse index inbred strain, the
segregating mutation causing an outlying phenotype relative to the index phenotype, the
method comprising the steps of:

e5 outcrossing at least one male animal of a mouse founder inbred strain to at least one
female animal of a mouse index inbred strain to obtain F1 progeny, the founder inbred strain
carrying random point mutations relative to a wild-type animal of the founder inbred strain,
the index inbred strain carrying a congenic dominant allele at a locus known to confer the
index phenotype and being genetically distinguishable from the founder inbred strain,

identifying one or more F₁ individuals displaying an outlying phenotype relative to the
index phenotype displayed by the index inbred strain, thereby indicating that at least one F₁
individual possesses an index phenotype-modifying mutation;

backcrossing gametes from male F1 progeny to at least one female of the index inbred
strain, with or without the index allele, to obtain N2 backcross progeny, wherein at least one
of the N2 backcross progeny that carry the dominant allele also exhibit the outlying
phenotype; and

verifying that the outlying phenotype is caused by a segregating single point mutation.

31. A method as claimed in Claim 30 wherein any of the crosses employ preserved

gametes.

32. A method as claimed in Claim 30 wherein the F₁ progeny and some of the N2 progeny exhibit an extreme outlying phenotype.

33. A method as claimed in Claim 32 wherein the segregating mutation is a heterozygous modifier of the index phenotype selected from a group consisting of an enhancing modifier and a suppressing modifier.

34. A method as claimed in Claim 30 wherein the dominant allele is a *Min* allele at an *Apc* locus in a mouse.

35. A method as claimed in Claim 30 wherein the index inbred strain and the founder inbred strain share an isogenic genetic background.

36. (Amended) A method as claimed in Claim 35 further comprising the step of mapping the segregating mutation using a mapping partner strain produced by the steps of:

treating an animal of the founder strain with a mutagenic agent to induce point mutations in the treated animal;

crossing the treated animal to an animal of the founder strain to produce F₁ progeny;
and

sib-mating F₁ and subsequent generation progeny until detrimental and lethal mutations are eliminated.

37. A method as claimed in Claim 30 wherein the founder inbred mouse strain is produced by a method comprising the step of treating a wild-type inbred mouse with a mutagenic agent to induce point mutations.

38. A method as claimed in Claim 37 wherein the mutagenic agent is ethylnitrosourea.

39. A method as claimed in Claim 35 wherein the method identifies a segregating mutation at a genetic locus that modifies tumor multiplicity in a C57BL/6 mouse congenic for the *Min* allele at the *Apc* locus, the method comprising the steps of:

outcrossing at least one male C57BL/6 mouse carrying random point mutations to a female C57BL/6 mouse congenic for the *Min* allele at the *Apc* locus to obtain F1 progeny, wherein at least one of the F1 progeny carries both the *Min* allele and a random point mutation; and

backcrossing gametes from male F1 progeny to at least one female C57BL/6 mouse congenic for the *Min* allele at the *Apc* locus to obtain N2 backcross progeny, wherein at least one of the N2 backcross progeny carries the *Min* allele and has a tumor multiplicity that is modified relative to tumor multiplicity in a C57BL/6 mouse congenic for the *Min* allele at the *Apc* locus, the modified tumor multiplicity being characteristic of the segregating mutation.

40. (Amended) A method as claimed in Claim 39 wherein the modified tumor multiplicity is evaluated according to a method comprising the steps of:

repeatedly applying for random permutations of mice among N2 backcross subkindreds a likelihood ratio test of the null hypothesis that no multiplicity modifier is segregating to obtain a p-value, wherein a p-value of less than 0.05 indicates a potential carrier of the segregating mutation;

when the p-value is less than 0.05, calculating, for each potential carrier that has offspring with information about tumor multiplicity, a LOD score for presence of the segregating mutation, wherein the LOD score is \log_{10} of a ratio of a first probability of offspring phenotype data if the potential carrier mouse carries a multiplicity modifier to a second probability of offspring phenotype data if the potential carrier mouse does not carry a multiplicity modifier, and wherein the second probabilities are calculated from an estimated background distribution and the first probabilities are calculated from a mixture of the estimated background distribution and an estimated modified distribution, where the estimated distributions are obtained by a maximum likelihood method; and

ranking the LOD scores of the potential carriers, whereby animals having the highest LOD scores are likely carriers of the segregating mutation.

41. A method as claimed in claim 39, further comprising the step of mapping the segregating mutation in the N2 backcross progeny using a mapping partner strain.

42. A method as claimed in Claim 41 wherein the mapping partner strain is produced by the steps of:

treating a C57BL/6 mouse with a mutagen to introduce random point mutations;

crossing the treated mouse to a C57BL/6 mouse to produce F1 progeny; and
sib-mating F1 and subsequent generation progeny until detrimental and lethal
mutations are eliminated.